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HIV-1 resistance to neutralizing antibodies: Determination of antibody concentrations leading to escape mutant evolution

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ABSTRACT

Broadly neutralizing antibodies against human immunodeficiency virus type 1 (HIV-1) are considered vital components of novel therapeutics and blueprints for vaccine research. Yet escape to even the most potent of these antibodies is imminent in natural infection. Measures to define antibody efficacy and prevent mutant selection are thus urgently needed. Here, we derive a mathematical framework to predict the concentration ranges for which antibody escape variants can outcompete their viral ancestors, referred to as mutant selection window (MSW). When determining the MSW, we focus on the differential efficacy of neutralizing antibodies against HIV-1 in two canonical infection routes, free-virus infection and cell–cell transmission. The latter has proven highly effective *in vitro* suggesting its importance for both *in vivo* spread as well as for escaping targeted intervention strategies. We observed a range of MSW patterns that highlight the potential of mutants to arise in both transmission pathways and over wide concentration ranges. Most importantly, we found that only when the arising mutant has both, residual sensitivity to the neutralizing antibody and reduced infectivity compared to the parental virus, antibody dosing outside of the MSW to restrict mutant selection is possible. Emergence of mutants that provide complete escape and have no considerable fitness loss cannot be prevented by adjusting antibody doses. The latter may in part explain the ubiquitous resistance to neutralizing antibodies observed in natural infection and antibody treatment. Based on our findings, combinations of antibodies targeting different epitopes should be favored for antibody-based interventions as this may render complete resistance less likely to occur and also increase chances that multiple escapes result in severe fitness loss of the virus making longer-term antibody treatment more feasible.

1. Introduction

During human immunodeficiency virus type 1 (HIV-1) infection, the neutralizing antibody response directed against the viral envelope (Env) proteins gp120 and gp41 co-evolves with the viral quasispecies (Frost et al., 2005; Overbaugh and Morris, 2012; Wibmer et al., 2013). In natural infection and antibody-based treatment, viral escape occurs even against the most potent broadly neutralizing antibodies (bnAb) highlighted by the fact that although bnAbs combine high potency and efficacy across divergent HIV-1 strains, they all have been isolated from individuals who acquired resistance and failed to control viremia (Klein et al., 2012; Trkola et al., 2005; Mascola and Haynes, 2013; Derdeyn et al., 2014; Caskey et al., 2015). Despite this, there is general agreement that bnAbs have an enormous potential in treatment and as blueprints for the design of protective vaccines provided that effective doses can be maintained *in vivo* (Burton et al., 2012; Schiffner et al., 2013).

In order to render antibody therapy successful, measures are needed to prevent or limit the evolution of neutralization resistant viral variants. Here, we present a framework to determine the sub-effective antibody concentration at which a resistant mutant can outcompete its viral ancestor on the population level. This concentration range, referred to as *mutant selection window (MSW)*, is crucial to judge whether treatment regimes can be optimized to prevent escape evolution. Determining this concentration range is, however, complicated by the virus' ability to transmit via two different routes. HIV-1, like many other enveloped viruses, can spread as cell-free virus particles or via cell–cell transmission. Virus spread via cell–cell transmission can occur through close-range cellular interactions either via pre-existing cellular contact zones such as the immunological synapse formed between dendritic cells and CD4 T-cells (McDonald et al., 2003; Arrighi et al., 2004; Piguet and

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Steinman, 2007) or short- and longer range actin-containing structures like filopodia (Lehmann et al., 2005; Sherer et al., 2007) and nanotubes (Sowinski et al., 2008). Best studied is the transmission via contact zones between HIV-1 infected and uninfected T cells, termed virological synapse (Jolly et al., 2004, 2007). This forms upon binding of the viral envelope protein to the CD4 receptor, leading to rapid recruitment and local concentration of Env, CD4, co-receptors, and adhesion molecules on the infected and target cells. While free-virus spread allows for transmission into more distant tissues, cell–cell transmission is thought to aid the virus to overcome physical and immunological barriers and has proven most effective *in vitro* in case of HIV-1 (Sattentau, 2008).

HIV-1 cell–cell transmission has been studied intensively *in vitro* using a variety of experimental assay systems that allowed the visualization and quantification of infection occurring through this route demonstrating that cell–cell transmission is far more efficient than free-virus spread (Gupta et al., 1989; Dimitrov et al., 1993; Jolly et al., 2004; Sherer et al., 2007; Sowinski et al., 2008; Hubner et al., 2009; Martin et al., 2010; Dale et al., 2011; Sigal et al., 2012; Abela et al., 2012; Permanyer et al., 2012a; Duncan et al., 2013; Malbec et al., 2013; Russell et al., 2013; Titanji et al., 2013; Zhong et al., 2013b, 2013; Agosto et al., 2014). One factor postulated to enhance efficacy is the clustering of receptors on effector cells in the virological synapse which allows for simultaneous infection by multiple virions (Rudnicka et al., 2009; Del Portillo et al., 2011; Russell et al., 2013). The improved infectivity in the cell–cell transmission pathway could be an important replication niche for unfit virus variants to gather compensating mutations that boost their replicative capacity (Brandenberg et al., 2014; Yen et al., 2014).

In addition to further experimental confirmation, new analytic and theory based strategies are needed to determine to which extent cell–cell transmission occurs *in vivo* and which role it plays for the establishment and spread of HIV-1 infection. How important it is to address these questions has been illustrated by *in vitro* studies revealing that antiretroviral therapy (ART) and broadly neutralizing antibodies (bnAb) do not neutralize HIV-1 cell–cell transmission with the same potency as free-virus spread (Poignard et al., 1999; Chen et al., 2007; Massanella et al., 2009; Sigal et al., 2012; Abela et al., 2012; Durham et al., 2012; Permanyer et al., 2012b; Malbec et al., 2013; Titanji et al., 2013; Agosto et al., 2014; McCoy et al., 2014; Reh et al., 2015). While the decreased efficiency of reverse transcriptase inhibitors observed *in vitro* can be partially explained by higher rates of virus transfer during cell–cell transmission (Sigal et al., 2012; Permanyer et al., 2012b; Duncan et al., 2013; Agosto et al., 2014), this appears not to be the only reason for the reduced neutralization capacity of bnAbs, entry inhibitors and other antiretroviral therapies (Sagar et al., 2012; Agosto et al., 2014). We recently showed that the neutralization efficiency of bnAbs during free-virus and cell–cell transmission is influenced by their neutralization kinetics: bnAbs were most potent against free-virus transmission when they showed a high activity prior to CD4 attachment of the virus, suggesting full access to their epitope on the virus Env and high binding affinity. On the contrary, bnAbs that displayed a decreased activity pre-attachment and during free-virus neutralization inhibited more potently after CD4 attachment of the virus and better retained their activity during cell–cell transmission (Reh et al., 2015). This is in accordance with previous time-of-addition (Martin et al., 2010) and post-attachment studies (Binley et al., 2003; Abela et al., 2012) and indicates that the functional characteristics of the antibody response to specific HIV-1 strains determine the neutralization capacity during free-virus and cell–cell transmission.

We recently analyzed the consequences of decreased inhibitory potentials of bnAbs during cell–cell transmission by comparing the probabilities that an antibody neutralization resistant viral variant arises via cell–cell and free-virus transmission (Reh et al., 2015). We showed that it is more likely for an escape mutant to arise during cell–cell transmission than during free-virus transmission. Thus, the cell–cell route could indeed serve as a rescue pathway for the virus, both against antiretroviral therapy and the antibody response.

Depending on the potency and type of antibody studied, escape even to broadly neutralizing antibodies can be rapid *in vivo* (Mascola et al., 2000; Trkola et al., 2005; Hessell et al., 2009, 2010; Moldt et al., 2012; Barouch et al., 2013; Halper-Stromberg et al., 2014; Klein et al., 2014; Doria-Rose et al., 2014; Caskey et al., 2015). A number of factors contribute to antibody neutralization efficacy *in vivo*, including tissue distribution, half-life, and the potential to elicit effector functions (Hessell et al., 2009; Bournazos et al., 2014; Ko et al., 2014; Trkola, 2014). Here, we seek to quantify the concentration ranges for which a specific antibody neutralization resistant variant can outcompete its viral ancestor because dosing to ensure inhibitory activity at levels that are above the limits of this MSW would be desirable. Determining the concentration ranges for which an antibody escape variant can outcompete its viral ancestor may help to understand the *in vivo* efficacy of neutralizing antibodies both as components of vaccines or as therapeutics. To this end, we extend the MSW theory (Baquero and Negri, 1997; Gullberg et al., 2011; Rosenbloom et al., 2012), to settings of HIV-1 neutralization by antibodies during free-virus and cell–cell infection. We calculate the MSW during free-virus and cell–cell infection as both Ab potency and virus infectivity can widely differ depending on the transmission pathway (Abela et al., 2012; Malbec et al., 2013; Zhong et al., 2013; McCoy et al., 2014; Brandenberg et al., 2014; Gombos et al., 2015; Reh et al., 2015). As we show here, the MSW of both pathways covers large concentration ranges. Definition of antibody doses above the critical range, the upper boundary of the MSW, is not possible in many cases, particularly when the emerging mutant has gained complete resistance to antibody inhibition without severe losses in fitness. In summary, our findings provide new insight into why escape to neutralizing antibodies is imminent in HIV-1 infection and highlight that neutralizing antibody therapy in the long term can only be effective if several antibodies are combined as a strategy to restrict rapid emergence of complete resistance combined with an antibody dosage above the upper border of the combined MSWs.

2. Materials and methods: studying antibody induced escape mutations

In this section we explain the experimental methods necessary to perform the mutant selection analysis. The derivation of the MSW model itself is laid out in Section 3.1.

2.1. General introduction to experimental methods

We and others (Mazurov et al., 2010; Abela et al., 2012; Janaka et al., 2013; Zhong et al., 2013; Reh et al., 2015) have recently established an assay system to exclusively analyze *in vitro* HIV-1 free-virus and cell–cell transmission. In short, Env pseudotyped virions are produced by a producer cell line. The pseudovirions are able to infect target cells only once (single-round). Upon infection, a luminescent marker is expressed through which infection of a target cell line can be monitored. To analyze neutralization, infection of the target cells is performed in the presence of increasing antibody concentration. We utilized two systems to distinguish cell–cell from free-virus transmission as recently described by (Reh et al., 2015): (i) To guarantee free-virus transmission, we only use the virions produced by the virus producer cell and add a catalyst which helps the virions to bind to and enter the target cells. (ii) To exclusively study cell–cell transmission, we transfer only the

Table 1
Overview of all bnAbs used in this study, reference of their first description and sources.

Inhibitor	Drug class	Target/Epitope	Reference	Source
b12	Antibody	gp120, CD4-bs	Barbas et al. (1992)	D. Burton, The Scripps Research Institute, La Jolla, USA
PG9	Antibody	gp120, V1V2 peptidoglycan	Walker et al. (2009)	D. Burton, The Scripps Research Institute, La Jolla, USA
PG16	Antibody	gp120, V1V2 peptidoglycan	Walker et al. (2009)	D. Burton, The Scripps Research Institute, La Jolla, USA
2G12	Antibody	Outer domain glycan	Trkola et al. (1996)	D. Katinger, Polymun, Vienna, Austria
PGT121	Antibody	gp120, V3 peptidoglycan	Walker et al. (2011)	D. Burton, The Scripps Research Institute, La Jolla, USA
PGT128	Antibody	gp120, V3 peptidoglycan	Walker et al. (2011)	D. Burton, The Scripps Research Institute, La Jolla, USA
2F5	Antibody	gp41, MPER (671-676)	Muster et al. (1993)	D. Katinger, Polymun, Vienna, Austria

infected producer cells and omit the catalyst to prevent free virions from binding to the target cells.

2.2. Reagents

Properties and sources of bnAbs used in this study are listed in Table 1. We thank D. Burton and D. Katinger for providing antibodies.

2.3. Cells

293-T cells were obtained from the American Type Culture Collection (ATCC), A3.01-CCR5 cells were described previously (Buttke and Folks, 1992; Abela et al., 2012). 293-T cells were cultivated in DMEM with 10% heat inactivated FCS and 1% Penicillin/Streptomycin. A3.01-CCR5 cells were maintained in RPMI with 10% heat inactivated FCS and 1% Penicillin/Streptomycin.

2.4. Viruses

Plasmids encoding the envelope of strains BG505 (subtype A), JR-FL, JR-CSF, SF162, REJO, AC10 (all subtype B) and ZM109 (subtype C) were obtained from the NIH ARP. Envelope (env) point mutations to achieve bnAb resistance (Table 2) were generated by site-directed mutagenesis (Agilent QuikChange II XL) according to the manufacturer instructions and sequenced by in-house Sanger sequencing to confirm presence of the desired mutations and absence of unintended sequence changes. For the production of single-round replicating pseudovirus stocks, 293-T cells were transfected with the respective viral pseudo typing constructs and env expression plasmids as described (Rusert et al., 2009). The following constructs were used: The luciferase reporter HIV-1 pseudotyped vector pNLlucAM (Rusert et al., 2011) (a gift from Dr. A. Marzosan) and the NL4-3 based pseudotyped vector with integrated inGluc reporter construct (NLinGluc (Janaka et al., 2013); a gift from Dr. M. Johnson).

2.5. Neutralization of cell-free Env-pseudotyped virus on A3.01-CCR5

Free-virus inhibition by bnAbs was assessed on A3.01-CCR5 cells using Env-pseudotyped NLlucAM reporter viruses. Virus stocks were harvested from 293-T 6-well cultures 48h post transfection and diluted 1:1 with culture medium (DMEM, 10% FCS, antibiotics). Viruses and serial dilutions of bnAbs were pre-incubated for 1h at 37 °C and added to 5×10^4 A3.01-CCR5 target cells in 96 well culture plates in the presence of 10 μg/ml diethylaminoethyl (DEAE, Amersham Biosciences, Connecticut, USA). After 65 h incubation at 37 °C, infection was assessed by luciferase reporter production after cell lysis and addition of firefly luciferase substrate (Promega, Madison, Wisconsin, USA). Emitted RLU were quantified on a Dynex MLX luminometer (Dynex Technologies Inc., Chantilly, Virginia, USA). The inhibitor concentrations causing 50% reduction in viral infectivity (50% inhibitory concentration; IC50) and the inhibition curve slopes were calculated by fitting pooled data from two to three independent experiments to sigmoid dose response curves (variable slope) using GraphPad Prism. For more details on the fitting algorithm please consult the Prism fitting guide (Graphpad Prism, 2015).

2.6. Assessing neutralization activity during cell–cell transmission of 293-T to A3.01-CCR5 cells

Neutralization of HIV-1 cell–cell transmission was analyzed on Env-pseudotyped NLinGluc virus transfected 293-T donor cells and A3.01-CCR5 target cells as recently described (Mazurov et al., 2010; Janaka et al., 2013; Zhong et al., 2013; Reh et al., 2015). The inGluc vector allows for distinction of cell–cell transmission from cell-fusion events. To exclusively study cell–cell transmission, free-virus infectivity was further restricted by the omission of DEAE in the infection media as previously described (Abela et al., 2012; Reh et al., 2015). For assessing neutralization of cell–cell transmission, 293-T cell 6-well cultures were transfected with *env* and NLinGluc plasmids in a 1:3 ratio. 6 h post transfection cells were collected, 5×10^3 cells seeded as infected donor cells per 96 well culture plate, and serial dilutions of inhibitors were added. After 1h incubation at 37 °C, 1.5×10^4 A3.01-CCR5 target cells in RPMI medium were added to the 293-T-inhibitor mix. After 65 h of incubation at 37 °C, Gaussia luciferase activity in the supernatant was quantified using the Renilla Luciferase Assay System (Promega, Madison Wisconsin, USA) according to the manufacturers' instructions. Neutralization data were analyzed with GraphPad Prism as described above.

2.7. Determination of entry fitness reduction of mutant strain envelopes in absence of antibodies, r

To derive an estimate for viral entry fitness in free-virus transmission, cell-free virus preparations of Env-pseudotyped NLlucAM reporter viruses were generated for all sensitive and resistant viruses using identical transfection conditions in 6-well plates. Infectivity of a set input (25 μl virus stock solution per 96-well) was then determined for all virus preparations in 2–3 independent experiments. Infectivity was measured in emitted RLU as quantified by the Dynex MLX luminometer (Dynex Technologies Inc., Chantilly, Virginia, USA). RLU values were averaged across all replicates in the independent experiments and used as estimate of entry fitness. Entry fitness during cell–cell transmission was determined in analogy using transfected 293-T cells instead of virus supernatant as input (5×10^3 transfected 293-T cells per 96-well). We obtained the fitness ratio of the mutant strain, *r*, by dividing the average RLU for the resistant by the one for the sensitive strain.

3. Results

3.1. A framework to study evolution of resistant viral variants in cell–cell versus free-virus spread

While we learned from earlier work that broadly neutralizing antibody escape variants of HIV-1 are overall more likely to arise during cell–cell than free-virus transmission (Reh et al., 2015),

Table 2
Overview over probed pairings of sensitive and resistant viral strains to neutralization of the designated antibody, their *IC*50s and slope values in cell–cell and free-virus transmission determined with GraphPad Prism, and the fitness ratio between antibody resistant and sensitive strain. The fitness ratio is measured in absence of antibodies and thus only depends on the particular sensitive and resistant strain as well as the transmission route (first three columns). If 50% inhibition was not reached with concentrations below 100 μg/ml the viral strain was denoted resistant to the respective antibody (100% res).

Sensitive strain (sen)	Resistant strain (res)	Antibody	Route	$IC50_{sen}$	m_{sen}	$IC50_{res}$	m_{res}	r
BG505 T332N	BG505	PGT121	fv	0.003727	0.7525	0.2572	0.6019	0.5387
BG505 T332N	BG505	PGT121	cc	0.3892	1.006	1.726	1	0.3632
BG505 T332N	BG505	PGT128	fv	0.003879	0.4772	1.928	0.7843	0.5387
BG505 T332N	BG505	PGT128	cc	0.4795	2.005	2.799	1.518	0.3632
JR-FLwt	JR-FL M373RP370L	b12	fv	0.002964	0.608	100% res	100% res	0.5287
JR-FLwt	JR-FL M373RP370L	b12	cc	0.3241	0.7493	100% res	100% res	0.3643
JR-FLwt	JR-FL N332S	2G12	fv	0.3213	0.6606	100% res	100% res	0.876
JR-FLwt	JR-FL N332S	2G12	cc	1.268	0.6218	100% res	100% res	0.8536
JR-FLwt	JR-FL D664N	2F5	fv	0.0685	0.4762	100% res	100% res	1.2957
JR-FLwt	JR-FL D664N	2F5	cc	1.225	0.7412	100% res	100% res	1.063
JR-FL E168KN189A	JR-FLwt	PG9	fv	0.04013	0.5142	100% res	100% res	1.4474
JR-FL E168KN189A	JR-FLwt	PG9	cc	0.4056	0.955	100% res	100% res	1.4911
JR-FL E168KN189A	JR-FLwt	PG16	fv	0.002178	0.4054	100% res	100% res	1.4474
JR-FL E168KN189A	JR-FLwt	PG16	cc	0.02982	0.5041	100% res	100% res	1.4911
REJOwt	REJO N160K	PG9	fv	0.03397	0.5473	100% res	100% res	0.6475
REJOwt	REJO N160K	PG9	cc	1.089	1.01	100% res	100% res	0.4927
ZM109wt	ZM109 N160K	PG9	fv	0.2887	0.4619	100% res	100% res	0.0636
ZM109wt	ZM109 N160K	PG9	cc	5.655	0.4091	100% res	100% res	0.4786
AC10wt	AC10 N160K	PG9	fv	0.2069	0.4802	100% res	100% res	0.4177
AC10wt	AC10 N160K	PG9	cc	0.9083	0.9159	100% res	100% res	0.7839
JR-CSFwt	JR-CSF N160K	PG9	fv	0.002547	0.6205	100% res	100% res	0.038
JR-CSFwt	JR-CSF N160K	PG9	cc	0.022	0.7148	100% res	100% res	0.1512
SF162 K160N	SF162wt	PG9	fv	0.004284	0.3252	100% res	100% res	0.2746
SF162 K160N	SF162wt	PG9	cc	0.2927	0.609	100% res	100% res	0.9747

concentration ranges for which a specific escape variant will arise and outcompete the sensitive strain have not yet been defined. We determine these ranges by applying a framework introduced for antibiotic killing of bacteria (Baquero and Negri, 1997; Gullberg et al., 2011) and later applied to drug-resistance evolution in HIV-1 infection (Rosenbloom et al., 2012). Of note, this framework does not make assumptions on whether the mutant strain is present upon drug delivery or arises through mutation in presence of a drug that is not 100% effective. For constructing the MSW, we compare the fitness of a virus' antibody neutralization sensitive (wild type) and Ab-resistant mutant strain. Here we use the term fitness in the sense of the replicative capacity which summarizes virus production and infection of new cells. Without neutralizing antibodies, the replicative capacity comprises only the viral baseline fitness, i.e. viral production and unhindered infection of new cells. If, however, neutralizing antibodies are present, the number of newly produced virions will be reduced through neutralization, resulting in a lower replicative capacity. Whether a mutant strain can outcompete its more sensitive viral ancestor thus depends on the reduction of the fitness by antibodies and in addition on the baseline fitness of both strains. All mathematical variables used in the following are summarized in Table 3.

Table 3
Parameter definitions.

Ab	Antibody
$IC50_{a,sen}$	Inhibitory concentration at which 50% of an antibody a's inhibitory potential is reached for the sensitive (wild type) virus
$m_{a,sen}$	Hill slope of inhibition curve measured for antibody a and a sensitive (wild type) strain
$c(Ab)$	Antibody concentration
$F_0^{a,sen}$	Fitness (replicative capacity) of the sensitive strain in absence of antibody a, following (Rosenbloom et al., 2012), we set this parameter to $F_0^{a,sen} = 10$
$F_0^{a,res}$	Fitness (replicative capacity) of the resistant strain (escape mutation) against antibody a, in absence of antibody a
r	Fitness reduction for mutant variant in absence of antibodies, i.e. $F_0^{a,res} = rF_0^{sen}$

We determine the neutralizing effect of one bnAb at different antibody concentrations in *in vitro* infectivity experiments (see Section 2 for details on the experimental system and Fig. 1A). The concentration at which 50% of the maximal inhibitory effect is reached, denoted by *IC50*, and the Hill-slope parameter of the inhibition curve, denoted by m, are obtained by fitting the Hill-curve (Chou, 1976) to the data:

$$\%\ \text{inhibition} = 100 \times \frac{c(Ab)^m}{c(Ab)^m + IC50^m} \tag{1}$$

where $c(Ab)$ denotes the antibody concentration. Eq. (1) directly translates into infectivity reduction, f_{inf}:

$$f_{\text{inf}}(c(Ab)) = 1 - \frac{c(Ab)^m}{c(Ab)^m + IC50^m} = \frac{1}{1 + (c(Ab)/IC50)^m} \tag{2}$$

Without loss of generality, we only explain the construction of the MSW in free-virus (fv) transmission. This mathematical framework can, however, also be applied to the cell–cell route by replacing the relevant measures by those obtained in the *in vitro* cell–cell (cc) inhibition assay. For one antibody, a, we measure its inhibitory potential against the sensitive viral strain, $v_{a,sen}$, and against the resistant antibody-escape mutant, $v_{a,res}$. We denote the corresponding *IC*50 and slope values with $IC50_{a,sen}$, $m_{a,sen}$ and $IC50_{a,res}$, $m_{a,res}$, respectively. The fitness of the sensitive strain in absence of antibodies is denoted by F_0^{sen}. As explained above, the viral fitness is a function of the antibody concentration. Using Eq. (2), the wild type fitness is

$$F^{a,sen}(c(Ab)) = F_0^{sen} f_{\text{inf}}(c(Ab)) = \frac{F_0^{sen}}{1 + (c(Ab)/IC50_{a,sen})^{m_{a,sen}}} \tag{3}$$

and for the mutant fitness we obtain:

$$F^{a,res}(c(Ab)) = \frac{F_0^{res}}{1 + (c(Ab)/IC50_{a,res})^{m_{a,res}}} = \frac{rF_0^{sen}}{1 + (c(Ab)/IC50_{a,res})^{m_{a,res}}} \tag{4}$$

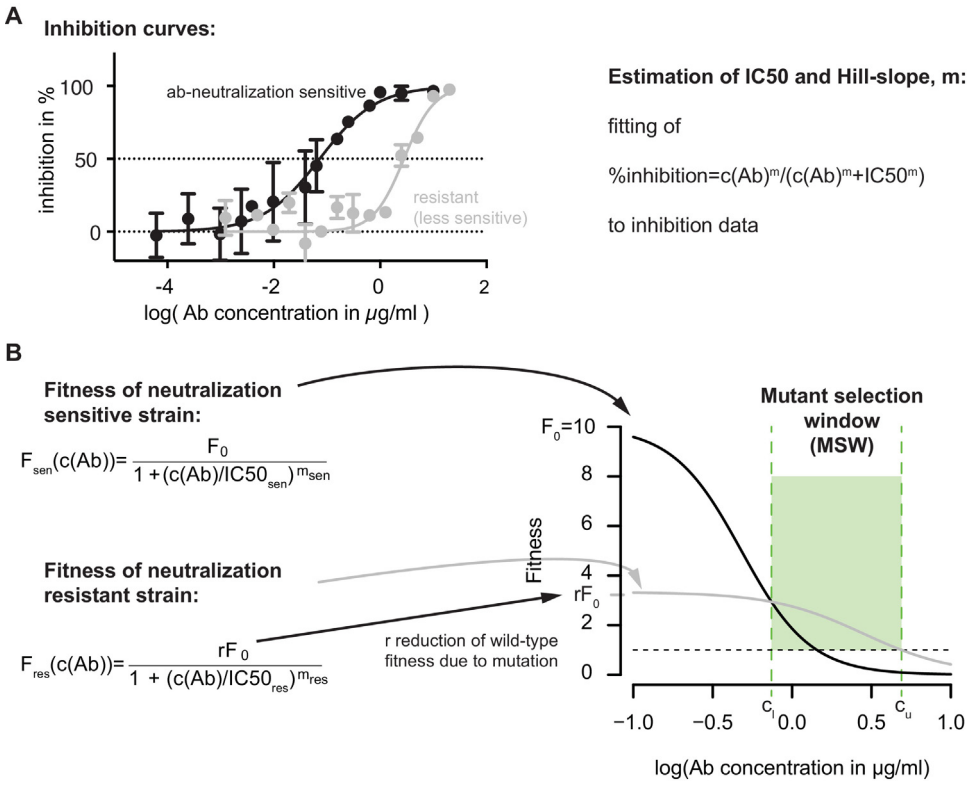

Fig. 1. Determination of the MSW for two related viral strains of which one is sensitive to antibody neutralization and the other is a less sensitive mutant variant. (A) The antibody inhibitory concentration at which 50% of the maximal effect is reached, IC50, determined in neutralization assays for both viral variants. (B) The fitness curves for the sensitive and the resistant strains are plotted. The MSW starts at the concentration c_l (green dashed line) at which the fitness of the resistant strain equals the fitness of the sensitive strain and ends at the concentration c_u (green dashed line) at which the fitness is smaller than 1.

with the fitness ratio $r := F_0^{res}/F_0^{sen}$. For estimating the MSW, we have to distinguish between two cases: (i) The mutation leads to decreased neutralization sensitivity. As a consequence, both IC50 and slope values can be derived from *in vitro* inhibition experiments (see Fig. 1A). (ii) The mutation leads to complete resistance and neither $IC50_{a,res}$ nor $m_{a,res}$ can be estimated out of the *in vitro* inhibition assays (see Fig. 2A).

(i) The mutation leads to decreased neutralization sensitivity. If the *IC*50 and the slope parameters for both sensitive and resistant viral strains can be determined, the mutant selection window is the concentration range for which the resistant mutant's fitness is bigger than the sensitive strain's fitness and bigger than 1. Only in this case, the resistant strain is able to outcompete the wild type strain on a population level. The lower bound of the MSW, c_l^a can theoretically be calculated by setting $F^{a,sen}(c(Ab)) = F^{a,res}(c(Ab))$ and solving this equation in respect to the antibody concentration, $c(Ab)$. However, this is mathematically only possible, if the slope parameter is an integer, in all other cases, the equation can only be solved

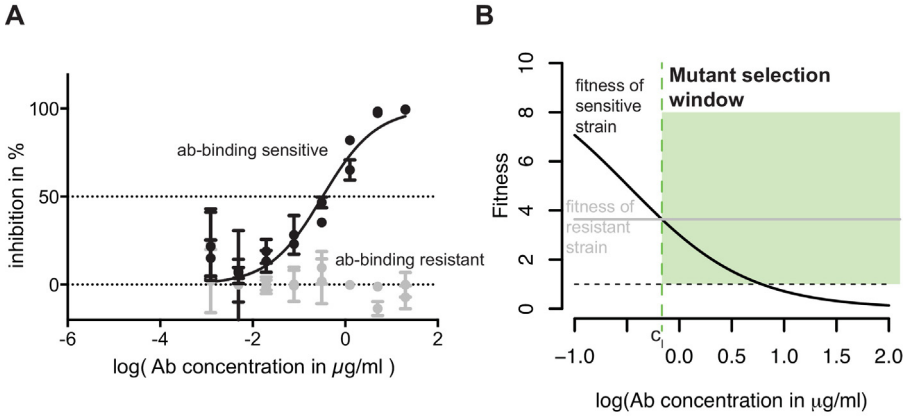

Fig. 2. Determination of the MSW for two genetically related viral strains for which a point mutation confers complete resistance to antibody neutralization. (A) The IC50 of the sensitive strain is determined by inhibition curves (black dots and fitted Hill-curve shown as black line). The inhibition curve of the resistant strain (gray dots) shows that the resistant strain is completely inert against antibody neutralization. (B) The fitness of the resistant strain (gray line) does not depend on the antibody concentration. Thus, the mutant selection window starts at the concentration c_l (green dashed line) at which the fitness of the sensitive strain is lower than the resistant strain.

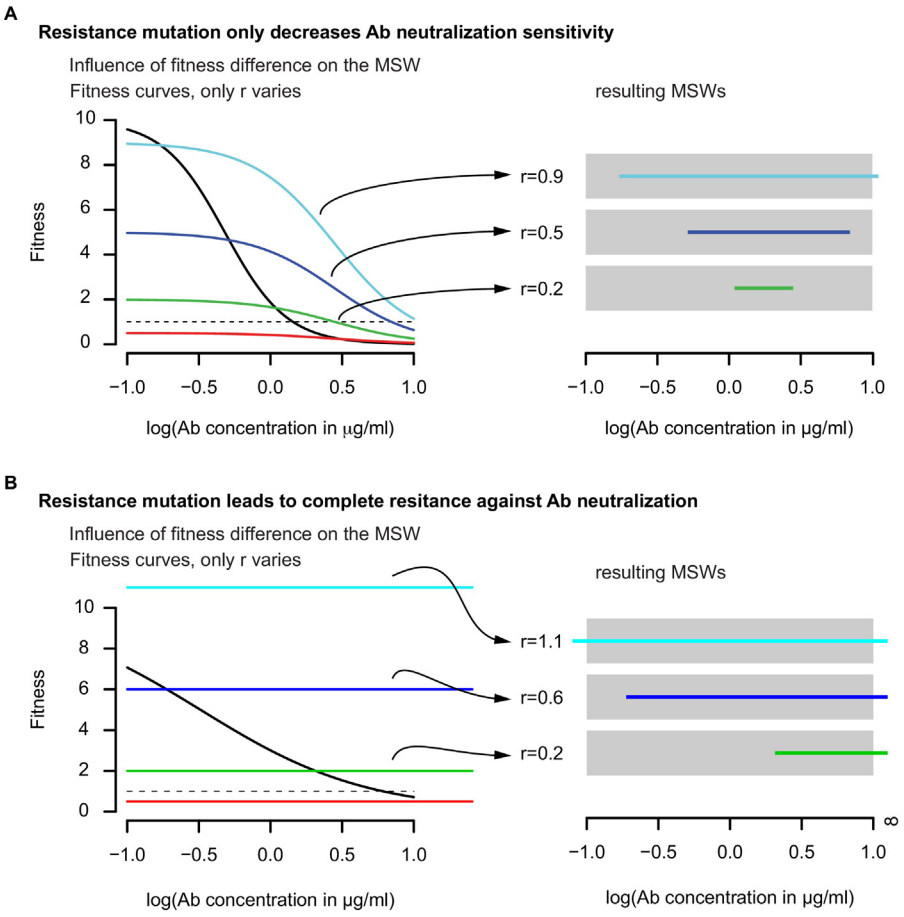

Fig. 3. Influence of the fitness ratio between Ab resistant and sensitive strain, r. In case (A) the resistant mutation decreases the neutralization sensitivity. In case (B), the resistant mutation leads to a completely resistant viral strain. The fitness ratio shapes the MSW and is therefore an important parameter to be experimentally determined.

numerically. The upper bound of the MSW, c_u^a can be calculated by solving $1 = F^{a,res}(c(Ab))$ in respect to the antibody concentration. Thus,

$$c_u^a = IC50_{a,res}(rF_0^{sen} - 1)^{1/m_{a,res}} \quad (5)$$

Fig. 1B shows a graphical illustration of how the MSW is derived.

(ii) The mutation leads to complete resistance. In the case the mutation makes the viral strain completely resistant against neutralization by antibody a, the viral strain's fitness is constant for all antibody concentrations, namely rF_0^{sen}. The lower bound of the MSW is therefore:

$$c_l^a = IC50_{a,sen}\left(\frac{1}{r} - 1\right)^{1/m_{a,sen}} \quad (6)$$

Whereas the upper bound is $c_u^a = \infty$. Fig. 2B shows a graphical illustration of this procedure.

All functions are coded in the R statistical language (R Core Team, 2014) and the scripts are available on request.

3.2. Influence of the fitness ratio between the Ab-sensitive ancestral strain and its escape mutant

The width of the MSW depends on the measured IC50s, the baseline fitness of the sensitive strain, F_0^{sen}, and the fitness ratio, r. Following Rosenbloom et al. (2012), we set the fitness to $F_0^{sen} = 10$ for every sensitive strain, which is based on an average of viral fitness measurements in the acute and chronic phase (Davey et al., 1999; Little et al., 1999; Ioannidis et al., 2000; Funk et al., 2001; Putter et al., 2002; Krakovska and Wahl, 2007; Ribeiro et al., 2010). However, the lower bound of the MSW is independent of the actual fitness value, F_0^{sen}. This is the case, because the lower bound is the antibody concentration at which sensitive and resistant strains' fitness values (Eqs. 3 and 4) are equal. When determining this concentration, F_0^{sen} cancels out (see also Eq. (6)). Only the upper bound positively correlates with F_0^{sen}, in case this bound is not infinite (Eq. (5)). This means that the fitness value F_0^{sen} only affects the upper bound of the MSW.

The fitness ratio r has a large impact on the width of the MSW. If the resistant strain only decreases the neutralization capacity of one antibody (case (i)), a small r (close to 0) leads to a small MSW (see Fig. 3A). If r is smaller than $1/F_0^{sen}$, the fitness of the mutant strain is below one and the strain cannot outcompete the sensitive strain for any antibody concentration. The closer r comes to 1, the wider the MSW becomes. If the resistant strain is completely insensitive to neutralization of the bnAb (case (ii), Fig. 3B), the lower bound of the MSW increases, the closer r comes to 0. If r is greater or equal to 1, the resistant mutant is fitter than the sensitive strain for all antibody concentrations. As r has such a big influence on the MSW, we experimentally determined r as described in Section 2.

3.3. MSW analysis reveals patterns of Ab induced selective pressure

Since bnAbs are considered as crucial components of HIV-1 vaccines as well as therapeutics (Walker and Burton, 2010; Kwong et al., 2011; Chen et al., 2013; Haynes and McElrath, 2013; Schiffner

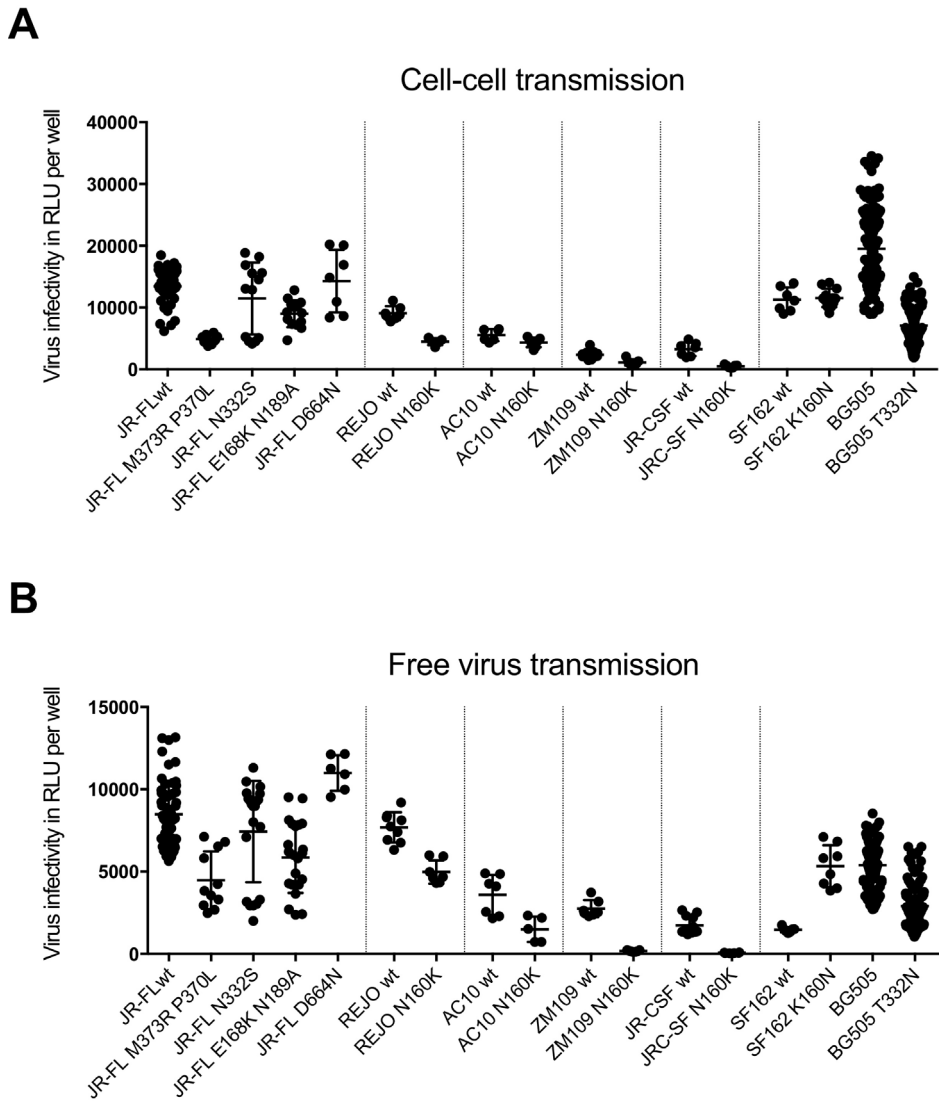

Fig. 4. Infectivity measurement of all viral strains in absence of antibodies. Infectivity of a set input (25 μl virus stock solution per 96-well) was determined for all virus preparations in 2–4 independent experiments with 3–4 replicates each. The mean of these infectivity values was used to determine the fitness ratio, *r*, between resistant and wild type viral strain by dividing the infectivity of the resistant by the infectivity of the sensitive strain. Table 2 summarizes all pairings of sensitive and resistant viral strains and their fitness ratios.

et al., 2013), and evolution of resistant viral strains will lead to their failure, we were particularly interested in determining the bnAb concentrations for which a resistant strain can outcompete its viral ancestor (mutant selection window, MSW). As we know from previous work, bnAb activity is generally lower during cell–cell than free-virus infection (Abela et al., 2012; Malbec et al., 2013; McCoy et al., 2014; Gombos et al., 2015; Reh et al., 2015) and mutants have a higher probability to emerge during cell–cell transmission (Reh et al., 2015). Hence, it is important to determine MSWs for both pathways to aid finding optimal *in vivo* doses outside these ranges to limit resistance evolution.

To this end, we determined the MSW of 12 pairings of genetically diverse HIV-1 strains (BG505 (subtype A), JR-FL, JR-CSF, SF162, REJO, AC10 (all subtype B) and ZM109 (subtype C)) against seven different bnAbs (b12, 2G12, PG9, PG16, PGT121, PGT128, 2F5). In each virus/bnAb combination, we compared an antibody sensitive and a related resistant strain encoding one to two resistance conferring point mutations. Our current analysis is a proof of principle study that strictly focuses on mutant selection of single antibody-virus strain pairs. While similar principles will apply for mutant selection *in vivo*, the complexity of determining the precise MSWs increases as it will be a combined effect of polyclonal antibody responses against diverse virus populations. We first derived the fitness ratio between sensitive and resistant strains. To estimate this ratio, we determined the efficacy of entry of Env pseudotyped viruses carrying either bnAb neutralization sensitive or resistant Envs in free-virus and cell–cell transmission (Fig. 4 and Table 2). Although our approach restricts the impact of fitness solely to differences in Env, this is a valid measure for the current analysis because only mutations in Env will occur in direct response to selective pressure exerted by antibodies. We next performed antibody inhibition experiments in free-virus and cell–cell transmission with both the sensitive and the resistant strain (Figs. 5–7). From these experimental data we obtained measures of IC50 and the Hill-slope parameter, *m* (Table 2). From these parameters, we then calculated and compared the MSW for each of the bnAb-sensitive/resistant virus pairings in free-virus and cell–cell transmission.

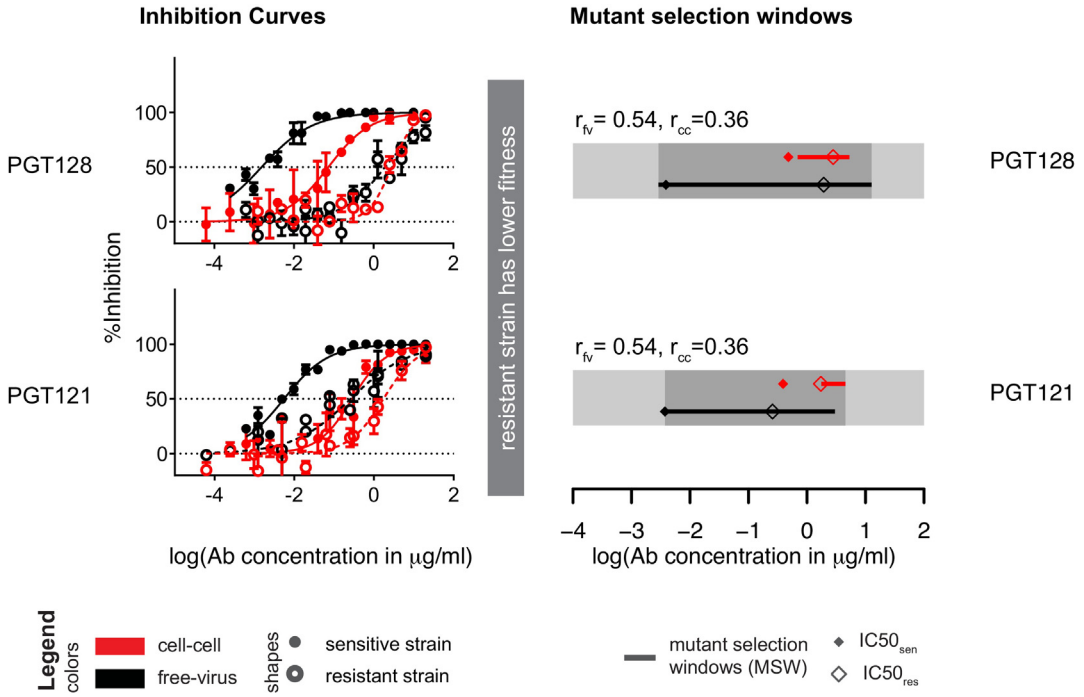

Fig. 5. Antibody inhibition curves and mutant selection windows for PGT121 and PGT128. The PGT121 and PGT128 sensitive strain BG505 T332N and the resistant BG505 strain are used as hypothetical escape pair to illustrate the impact of the MSW of the bnAbs PGT121 and PGT128. Black symbols refer to free-virus spread and red symbols refer to cell–cell spread. The gray shaded areas mark the displayed antibody concentration and the dark gray shaded area displays the combined MSW for free-virus and cell–cell transmission.

3.3.1. Determining the MSW for resistance mutations providing partial decrease in neutralization sensitivity

We first applied our theory to a situation where the resistant strain has partially lost its neutralization sensitivity. BG505 is relatively resistant to neutralization by the two V3 crown glycan dependent bnAbs PGT121 and PGT128 reflected by the comparatively high IC50 concentrations needed to block this strain (free-virus and cell–cell transmission IC50 of 0.26/1.7 and 1.9/2.8 μg/ml, respectively). Resistance in BG505 is inferred by loss of the N-glycosylation site at amino acid position 332 which is crucial for several bnAbs including PGT121, PGT128 and 2G12 (Sanders et al., 2002; Scanlan et al., 2002; Calarese et al., 2003; Pejchal et al., 2011; Julien et al., 2013; Sok et al., 2014). A variant with a functional N332 site, BG505 T332N, has a restored sensitivity to PGT121 and PGT128 (free-virus and cell–cell transmission IC50 of 0.0037/0.39 and 0.0039/0.48 μg/ml for PGT121 and PGT128, respectively). We thus used the sensitive strain BG505 T332N and the resistant BG505 strain as hypothetical escape pair to illustrate the impact of the MSW of the bnAbs PGT121 and PGT128 in this setting. Importantly, we found that the Env of BG505 is less fit in comparison to BG505 T332N with a fitness ratio of $r = 0.33$. In Fig. 5 we show the mutant selection windows for the sensitive strain BG505 T332N and its resistant mutant variant BG505 as a function of the antibody concentrations of PGT121 and PGT128 together with the measured IC50 values. The direct comparison of the MSWs in both free-virus and cell–cell transmission of the two Abs in these settings revealed that while the MSW of both pathways was overall in a comparable range for both bnAbs (within 1 order of magnitude), there were bnAb-dependent differences. PGT128 had a relatively wide MSW for free-virus (0.0029– 12.7 μg/ml) which overlaps with this of cell–cell transmission (0.69–5.29 μg/ml). PGT121, however, has almost non-overlapping MSWs in the two transmission pathways. Of note, the actual IC50 values of the two Abs in the two transmission modes do not provide information on the respective MSWs, highlighting the importance to assess the MSW specifically. When the resistant mutation does not lead to a complete loss in neutralization activity, antibody treatment without risking that a specific resistance mutation arises is possible, provided that the chosen Ab concentration is higher than the upper bound of the MSW for both, free-virus and cell–cell transmission. However, doses that are required can be quite high: In the example depicted in Fig. 5, concentrations of more than 10 μg/ml of PGT121 would be required to suppress mutant selection *in vitro*.

3.3.2. Complete resistance: one virus tested against different antibodies

During the course of an HIV-1 infection, the immune system produces a vigorous polyclonal antibody response to the envelope trimer. Thus, viral strains are under selective pressure exerted by many different antibody specificities. To mimic such a situation, we tested five different broadly neutralizing antibodies (b12, 2G12, 2F5, PG9 and PG16) against various JR-FL variants that either confer sensitivity (PG9 and PG16) or resistance (b12, 2G12, 2F5) compared to the parental JR-FL strain (Fig. 6, Table 2). In all combinations probed, the resistant variant was a complete Ab escape variant. In the cases of b12- and 2G12- sensitive/resistant viral strains, the resistant strains had lower relative fitness values ($r < 1$) than the sensitive strain in both transmission pathways. In the cases of 2F5-, PG9- and PG16-sensitive/resistant viral strains, the resistant strain had higher fitness ($r > 1$) (Table 2). Fig. 6 shows that complete resistance of the arising mutation has a huge impact on the width of the MSW. There is no residual effect of the Ab on the mutant virus and this variant can therefore evolve and outcompete its sensitive viral ancestor at any antibody concentration higher than the lower bound of the MSW. Considering that inhibition in free-virus is more effective (reflected by lower IC50 values), the MSW for free-virus inhibition can cover a wider concentration range (starting already at lower Ab concentration) than the MSW for cell–cell transmission, as e.g. seen for 2F5 and 2G12. In several of the studied virus pairings, the mutant virus proved to have higher entry fitness. While *in vivo*

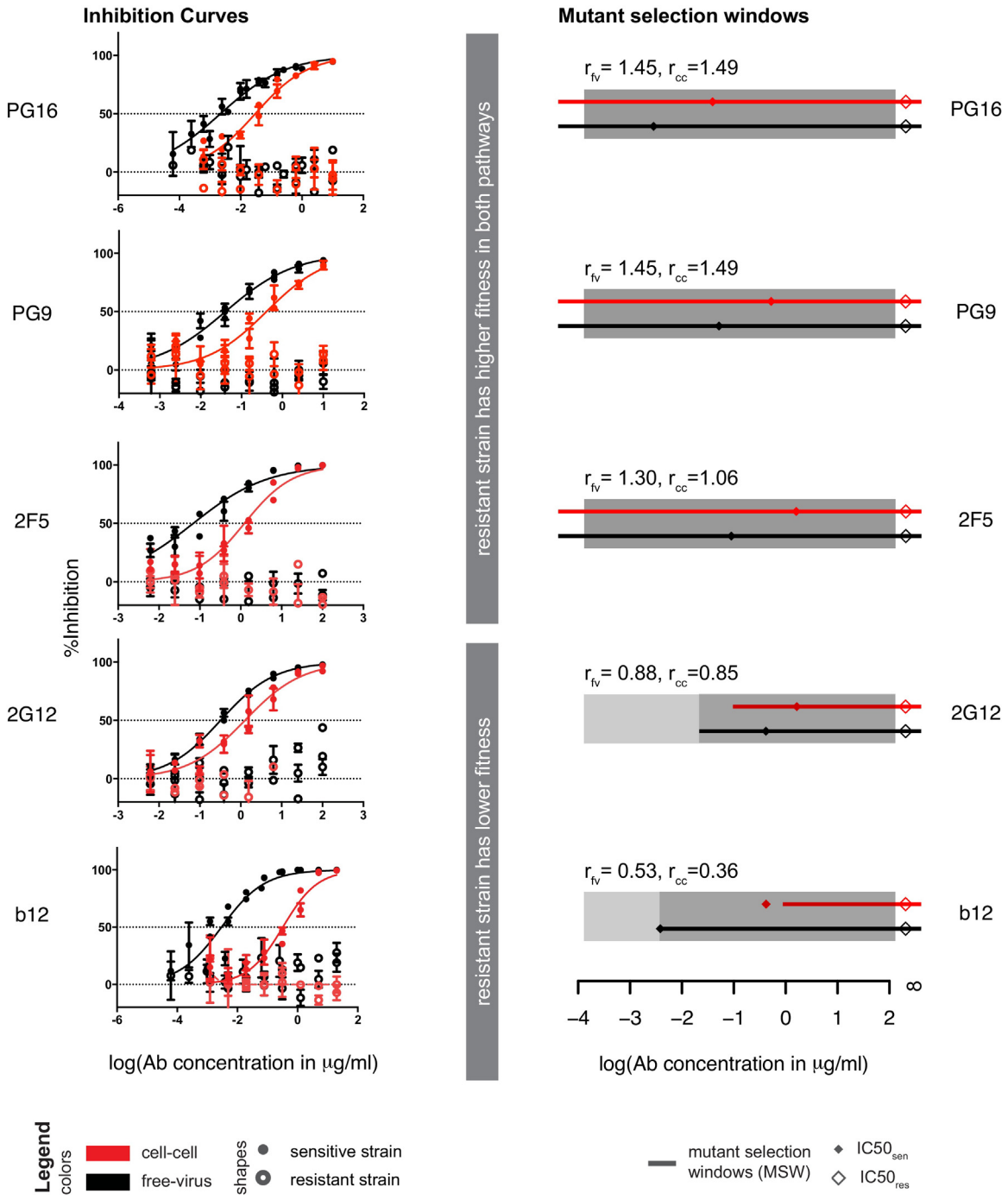

Fig. 6. Antibody inhibition curves and mutant selection window for JR-FL variants against five different antibodies determined for free-virus (black symbols) and cell–cell (red symbols) transmission. The gray shaded areas mark the displayed antibody concentration and the dark gray shaded area displays the combined MSW for free-virus and cell–cell transmission.

initially emerging mutations are likely less fit than the parental strains, increases in fitness may nevertheless occur in the course of accumulating replication compensating mutations. In the scenarios where the resistant mutant has higher fitness (bnAbs 2F5, PG9, PG16), the MSW covers the entire range of possible antibody concentrations.

3.3.3. *Complete resistance: comparative analysis of one bnAb against divergent viral strains*

Antibodies as components of vaccines or therapeutics need to be active against genetically diverse viruses of different subtypes as they face different inocula compositions and high within-host diversity, respectively. We therefore compared the MSWs of one antibody, PG9, against diverse viruses (Fig. 7 and Table 2). The N-glycosylation site N160 is crucial for PG9 activity and for the functional integrity of the virus envelope of many strains, as loss of N160 is frequently associated with severely decreased infectivity (Brandenberg et al., 2014). The wild type virus strains REJO, ZM109, AC10 and JR-CSF are sensitive to PG9 neutralization. Upon changing the asparagine to lysine at position 160, the strains become completely resistant to PG9 neutralization. The SF162 wild type strain already carries a PG9 resistance conferring lysine at position 160 such that we introduced an asparagine at this position to obtain a matching SF162 viral strain sensitive to PG9 neutraliza-

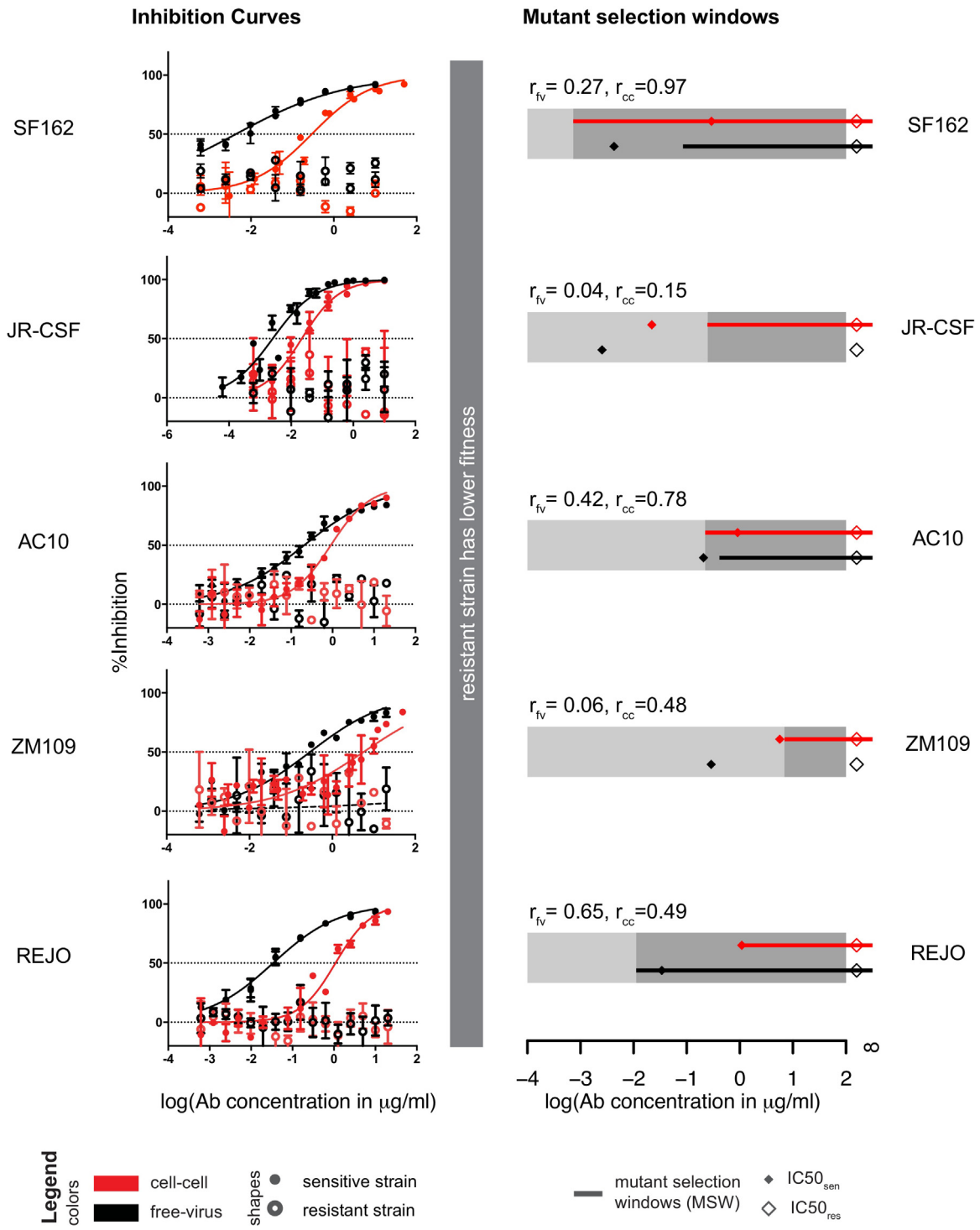

Fig. 7. Inhibition curves and mutant selection windows of PG9-sensitive and resistant viral strain pairs descending from five different HIV-1 viruses determined for free-virus (black symbols) and cell–cell (red symbols) transmission. The gray shaded areas mark the displayed antibody concentration and the dark gray shaded area displays the combined MSW for free-virus and cell–cell transmission. (For interpretation of reference to color in this figure legend, the reader is referred to the web version of this article.)

tion. We used Eq. (6) to determine the lower bound of the MSW, as all probed mutant strains were completely resistant against PG9. Fig. 7 shows that the MSWs are quite diverse and how much these ranges are governed by the fitness ratio. If a 100% resistance conferring mutant has an infectivity comparable to the sensitive parental virus (e.g. SF162 during cell–cell transmission, $r = 0.9747$), the MSW is extremely wide. Therefore, the mutant can be selected for and survive at essentially any antibody concentration. For viral strains that do not have such an extreme MSW, the MSW of free-virus and cell–cell transmission should be considered to prevent a resistant viral variant to arise via either route, when the bnAb is used as treatment. However, it is important to consider that the antibodies can have substantially different efficacies in the cell–cell and free-virus transmission pathways. For ZM109 and JR-CSF, the neutralization resistant viral strains had such low fitness values in free-virus transmission that replication and therefore the evolution of the particular Ab resistant mutant can only occur in cell–cell transmission. In most cases, the MSW for cell–cell

transmission is wider than for free-virus transmission (Fig. 7). However, the MSW of REJO inhibited by PG9 in free-virus transmission starts at a lower concentration than for cell–cell transmission. This finding highlights the necessity to determine the MSW for both transmission routes. In sum, our results reveal that it is unlikely to reach effective concentrations for single bnAb treatments *in vivo* that will reliably prevent resistant strains to emerge as long as point mutations can evolve that provide complete resistance without any major fitness cost.

4. Synthesis, discussion and outlook

Any antiviral treatment strategy must reduce viral loads but also prevent treatment failure caused by the evolution of treatment resistant viral strains. Broadly neutralizing antibodies against HIV-1 which are considered lead components of novel therapeutic approaches and vaccines (Burton et al., 2012; Schiffner et al., 2013), have different neutralization potencies in free-virus and cell–cell transmission (Abela et al., 2012; Malbec et al., 2013; McCoy et al., 2014; Gombos et al., 2015; Reh et al., 2015). Therefore, both pathways need to be considered when assessing the efficacy of antibody treatment. In the current study, we determined the potential impact of the two transmission pathways in fostering the occurrence of virus strains that acquired resistance mutations conferring partial or complete resistance to antibody neutralization. In particular, we investigated whether concentration ranges exist that prevent mutant selection in both pathways, hence, allowing optimal dosing of therapies. To this end, we extended the framework of mutant selection windows (Baquero and Negri, 1997; Rosenbloom et al., 2012) to determine the antibody concentration ranges for which a specific antibody escape variant can outcompete its ancestral strain. To obtain the crucial parameters for the MSW framework, we measured the inhibitory potential of broadly neutralizing antibodies against neutralization sensitive and resistant viral strains in assay systems that allow for separation of cell–cell from free-virus transmission *in vitro*.

In contrast to our previous work (Reh et al., 2015), where we showed that an observed mutant variant has more likely arisen during the cell–cell pathway, we determined here the concentration ranges for which an escape variant can outcompete its viral ancestor. When these MSWs for free-virus and cell–cell transmission overlap, the escape variant can arise via both transmission routes. However, if the MSW for cell–cell transmission is wider than the MSW for free-virus transmission, the cell–cell route may serve as a rescue pathway for the virus to evolve resistant viral variants.

Across all viruses and antibodies probed, we saw that MSWs for both transmission pathways are generally very wide. Thus, it will be difficult, if not impossible, to determine concentration ranges that prevent mutant selection *in vivo*. Our findings are in line with what we know from antibody efficacy in established HIV-1 infection where escape to single antibody pressure occurs univocally unless antibodies are highly potent and present at doses orders of magnitude higher than their *in vitro* effective concentrations (Frost et al., 2005; Trkola et al., 2005; Klein et al., 2012; Overbaugh and Morris, 2012; Barouch et al., 2013; Wibmer et al., 2013; Caskey et al., 2015). Our study provides additional proof that combining different antibody specificities in a treatment regime will be crucial because multiple escapes will most likely be more difficult to achieve and these mutations will in most cases come with a strong fitness cost for the virus. Indeed, as we showed here, the impact of the fitness ratio between the sensitive and the resistant strain proved decisive for the width of the MSW. Hence, treatments for which escape mutations severely impact infectivity are of tremendous advantage.

It will be intriguing to extend the MSW framework to include further biologically relevant parameters. In addition to studying antibody inhibition that reaches 100% efficacy, it may be important to explore antibodies with lower efficacy, i.e. with activity plateaus below 100%. Such scenarios can be integrated into our framework by adapting the infectivity reduction in Eqs. (3) and (4). Antibody concentrations can vary over time and also between body compartments. Similar to a framework introduced for drug induced escape mutant strains (Rosenbloom et al., 2012), the MSW framework can be extended to predict the timing of the occurrence of a viral escape mutant upon selective pressure induced by antibodies. To this end, the exact sequence of the wild type and mutant strain must be known to determine the probability that the mutant strain can arise during reverse transcription. By taking into account neutralization levels, the mutation probability can then be used in combination with a virus dynamics model to predict the dynamics of the wild type and mutant on a population level. However, unlike drug resistance where often a single or very few mutations are sufficient to infer resistance, escape to neutralizing antibodies is much more complex as the envelope has ample possibilities of evading neutralizing antibodies. Besides mutating the antibody's binding site, the Env can evade antibody recognition by altering its shielding properties (e.g. by modification of the V1V2 loop), by increasing or alternating the glycan shield and by introducing mutations at distant sites in the envelope that induce conformational shifts that either affect access of the antibody epitope or the conformation of the antibody's binding site itself (Rusert et al., 2011; Kwong et al., 2011).

Our current study is a first step towards defining the impact of the MSW. Here we relied solely on *in vitro* assays using engineered cell lines and Env-pseudotyped viruses. The fitness measurements we made are therefore only referring to differences in entry capacity of the virus strains. Assessment of *in vivo* derived strains which will harbor a range of mutations that shape fitness also in unrelated genes will be important to include in future studies. The resistance mutations we assessed are specific point mutations known to eliminate the respective antibody epitopes. However, the *in vivo* mutant selection process will be iterative involving several rounds of acquiring resistance conferring mutations and compensating mutations that improve fitness. As laid out in Section 3.2, the fitness of the sensitive strain only impacts the upper but no the lower bound of the MSW and the ratio between sensitive and resistant strains' fitness has a tremendous impact on the MSW. Both quantities need to be determined individually for an exact determination of the MSWs. Hence, determining the *in vivo* MSWs will be more complex and will need challenging longitudinal assessment of emerging viruses and autologous neutralization responses (Trkola et al., 2008; van Gils and Sanders, 2014).

It is important to note, that the MSWs defined here are solely based on *in vitro* experiments, with their intrinsic assay limitations of cell type used and maximal antibody concentrations. While similar relationships between infectivity and efficacies of antibodies in free-virus and cell–cell transmission can be assumed *in vivo*, the doses determined for MSWs in the present study cannot be directly transferred to *in vivo* situations where other factors like tissue distribution and half-life of the mAb and contribution of antibody effector functions also come into play. It is, however, intriguing that the upper bounds of the MSW we determined here are orders of magnitude higher than the *in vitro* determined IC50 values which compares well with the discrepancy between *in vitro* and *in vivo* effective antibody titers described to be 100–1000-fold higher (Parren et al., 2001; Trkola et al., 2005, 2008; Hessell et al., 2009; Moldt et al., 2012; Reh et al., 2015). While these high *in vivo* effective concentrations cannot be tested *in vitro*, *in vitro* measured inhibitory activities correlate with the *in vivo* concentrations (van Gils and Sanders, 2014).

Intriguingly, the MSW can be even wider in a setting where the antibody has less efficacy (e.g. during cell–cell transmission).

This is most often the case, when the difference between antibody efficacy for the sensitive and resistant strain is less pronounced in free-virus in comparison to cell–cell transmission. Detailed studies on the efficacy of neutralization during cell–cell transmission especially those considering the stoichiometry of entry and neutralization will be crucial to define the underlying mechanisms of efficacy loss.

Based on our findings, we suggest to consider the MSW when analyzing or even predicting the outcome of passive immunization studies currently conducted with bnAbs to prevent or treat HIV-1 infection (Mascola et al., 2000; Trkola et al., 2005; Hessell et al., 2009, 2010; Moldt et al., 2012; Barouch et al., 2013; Halper-Stromberg et al., 2014; Klein et al., 2014; Doria-Rose et al., 2014; Caskey et al., 2015). This may provide valuable information towards neutralizing antibody efficacy *in vivo* and the processes that lead to within-host mutant selection through free-virus and cell–cell transmission.

Our analysis highlights the difficulties in dose finding for neutralizing antibody therapy and provides novel insights why neutralization resistance selection in HIV-1 infection may be so effective. As we show here, both free-virus and cell–cell transmission provide possibilities for specific virus mutants to emerge over large antibody concentration ranges. Hence, strategies are needed to limit the MSW to narrow concentration ranges and to increase antibody pressure in a way that resistance mutations come with severe fitness costs. Combination therapy with several antibodies targeting different epitopes on the virus envelope may be one way to achieve this goal.

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References

Abela, I.A., Berlinger, L., Schanz, M., Reynell, L., Günthard, H.F., Rusert, P., Trkola, A., 2012. Cell–cell transmission enables HIV-1 to evade inhibition by potent CD4bs directed antibodies. PLoS Pathog. 8, e1002634.

Agosto, L.M., Zhong, P., Munro, J., Mothes, W., 2014. Highly active antiretroviral therapies are effective against HIV-1 cell-to-cell transmission. PLoS Pathog. 10, e1003982.

Arrighi, J.F., Pion, M., Garcia, E., Escola, J.M., van Kooyk, Y., Geijtenbeek, T.B., Piguet, V., 2004. DC-SIGN-mediated infectious synapse formation enhances X4 HIV-1 transmission from dendritic cells to T cells. J. Exp. Med. 200, 1279–1288.

Baquero, F., Negri, M.C., 1997. Strategies to minimize the development of antibiotic resistance. J. Chemother. 9, 29–37.

Barbas III, C.F., Bjorling, E., Chiodi, F., Dunlop, N., Cababa, D., Jones, T.M., Zebedee, S.L., Persson, M.A.A., Nara, P.L., Norrby, E., Burton, D.R., 1992. Recombinant human fab fragments neutralize human type 1 immunodeficiency virus in in vitro. Proc. Natl. Acad. Sci. U. S. A. 89, 9339–9343.

Barouch, D.H., Whitney, J.B., Moldt, B., Klein, F., Oliveira, T.Y., Liu, J., Stephenson, K.E., Chang, H.W., Shekhar, K., Gupta, S., Nkolola, J.P., Seaman, M.S., Smith, K.M., Borducchi, E.N., Cabral, C., Smith, J.Y., Blackmore, S., Sanisetty, S., Perry, J.R., Beck, M., Lewis, M.G., Rinaldi, W., Chakraborty, A.K., Poignard, P., Nussenzweig, M.C., Burton, D.R., 2013. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature 503, 224–228.

Binley, J.M., Cayanan, C.S., Wiley, C., Schulke, N., Olson, W.C., Burton, D.R., 2003. Redox-triggered infection by disulfide-shackled human immunodeficiency virus type 1 pseudovirions. J. Virol. 77, 5678–5684.

Bournazos, S., Klein, F., Pietzsch, J., Seaman, M.S., Nussenzweig, M.C., Ravetch, J.V., 2014. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. Cell 158, 1243–1253.

Brandenberg, O.F., Rusert, P., Magnus, C., Weber, J., Böni, J., Günthard, H.F., Regoes, R.R., Trkola, A., 2014. Partial rescue of V1V2 mutant infectivity by HIV-1 cell–cell transmission supports the domain's exceptional capacity for sequence variation. Retrovirology, 1–22.

Burton, D.R., Ahmed, R., Barouch, D.H., Butera, S.T., Crotty, S., Godzik, A., Kaufmann, D.E., McElrath, M.J., Nussenzweig, M.C., Pulendran, B., Scanlan, C.N., Schief, W.R., Silvestri, G., Streeck, H., Walker, B.D., Walker, L.M., Ward, A.B., Wilson, I.A., Wyatt, R., 2012. A blueprint for HIV vaccine discovery. Cell Host Microbe 12, 396–407.

Buttke, T.M., Folks, T.M., 1992. Complete replacement of membrane cholesterol with 4,4′,14-trimethyl sterols in a human T cell line defective in lanosterol demethylation. J. Biol. Chem. 267, 8819–8826.

Calarese, D.A., Scanlan, C.N., Zwick, M.B., Deechongkit, S., Mimura, Y., Kunert, R., Zhu, P., Wormald, M.R., Stanfield, R.L., Roux, K.H., Kelly, J.W., Rudd, P.M., Dwek, R.A., Katinger, H., Burton, D.R., Wilson, I.A., 2003. Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. Science 300, 2065–2071.

Caskey, M., Klein, F., Lorenzi, J.C.C., Seaman, M.S., West, A.P., Buckley, N., Kremer, G., Nogueira, L., Braunschweig, M., Scheid, J.F., Horwitz, J.A., Shimeliovich, I., Ben-Avraham, S., Witmer-Pack, M., Platten, M., Lehmann, C., Burke, L.A., Hawthorne, T., Gorelick, R.J., Walker, B.D., Keler, T., Gulick, R.M., Fätkenheuer, G., Schlesinger, S.J., Nussenzweig, M.C., 2015. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature 522, 487–491.

Chen, P., Hubner, W., Spinelli, M.A., Chen, B.K., 2007. Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. J. Virol. 81, 12582–12595.

Chen, W., Ying, T., Dimitrov, D.S., 2013. Antibody-based candidate therapeutics against HIV-1: implications for virus eradication and vaccine design. Expert Opin. Biol. Ther. 13, 657–671.

Chou, T., 1976. Deviation and properties of Michaelis–Menten type and hill type equations for reference ligands. J. Theor. Biol. 59, 253–276.

Dale, B.M., McNerney, G.P., Thompson, D.L., Hubner, W., de Los Reyes, K., Chuang, F.Y., Huser, T., Chen, B.K., 2011. Cell-to-cell transfer of HIV-1 via virological synapses leads to endosomal virion maturation that activates viral membrane fusion. Cell Host Microbe 10, 551–562.

Davey, R.T., Bhat, N., Yoder, C., Chun, T.W., Metcalf, J.A., Dewar, R., Natarajan, V., Lempicki, R.A., Adelsberger, J.W., Miller, K.D., Kovacs, J.A., Polis, M.A., Walker, R.E., Falloon, J., Masur, H., Gee, D., Baseler, M., Dimitrov, D.S., Fauci, A.S., Lane, H.C., 1999. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc. Natl. Acad. Sci. U. S. A. 96, 15109–15114.

Del Portillo, A., Tripodi, J., Najfeld, V., Wodarz, D., Levy, D.N., Chen, B.K., 2011. Multiploid inheritance of HIV-1 during cell-to-cell infection. J. Virol. 85, 7169–7176.

Derdeyn, C.A., Moore, P.L., Morris, L., 2014. Development of broadly neutralizing antibodies from autologous neutralizing antibody responses in HIV infection. Curr. Opin. HIV AIDS 9, 210–216.

Dimitrov, D.S., Willey, R.L., Sato, H., Chang, L.J., Blumenthal, R., Martin, M.A., 1993. Quantitation of human immunodeficiency virus type 1 infection kinetics. J. Virol. 67, 2182–2190.

Doria-Rose, N.A., Schramm, C.A., Gorman, J., Moore, P.L., Bhiman, J.N., DeKosky, B.J., Ernandes, M.J., Georgiev, I.S., Kim, H.J., Pancera, M., Staupe, R.P., Altae-Tran, H.R., Bailer, R.T., Crooks, E.T., Cupo, A., Druz, A., Garrett, N.J., Hoi, K.H., Kong, R., Louder, M.K., Longo, N.S., McKee, K., Nonyane, M., O'Dell, S., Roark, R.S., Rudicell, R.S., Schmidt, S.D., Sheward, D.J., Soto, C., Wibmer, C.K., Yang, Y., Zhang, Z., Program, N.C.S., Mullikin, J.C., Binley, J.M., Sanders, R.W., Wilson, I.A., Moore, J.P., Ward, A.B., Georgiou, G., Williamson, C., Abdool Karim, S.S., Morris, L., Kwong, P.D., Shapiro, L., Mascola, J.R., 2014. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature 509, 55–62.

Duncan, C.J., Russell, R.A., Sattentau, Q.J., 2013. High multiplicity HIV-1 cell-to-cell transmission from macrophages to CD4+ T cells limits antiretroviral efficacy. AIDS 27, 2201–2206.

Durham, N.D., Yewdall, A.W., Chen, P., Lee, R., Zony, C., Robinson, J.E., Chen, B.K., 2012. Neutralization resistance of virological synapse-mediated HIV-1 Infection is regulated by the gp41 cytoplasmic tail. J. Virol. 86, 7484–7495.

Frost, S.D.W., Wrin, T., Smith, D.M., Pond, S.L.K., Liu, Y., Paxinos, E., Chappey, C., Galovich, J., Beauchaine, J., Petropoulos, C.J., Little, S.J., Richman, D.D., 2005. Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. Proc. Natl. Acad. Sci. U. S. A. 102, 18514–18519.

Funk, G.A., Fischer, M., Joos, B., Opravil, M., Gunthard, H.F., Ledergerber, B., Bonhoeffer, S., 2001. Quantification of in vivo replicative capacity of HIV-1 in different compartments of infected cells. JAIDS J. Acquired Immune Deficiency Syndromes 26, 397–404.

van Gils, M.J., Sanders, R.W., 2014. In vivo protection by broadly neutralizing hiv antibodies. Trends Microbiol. 22, 550–551.

Gombos, R.B., Kolodkin-Gal, D., Eslamizar, L., Owuor, J.O., Mazzola, E., Gonzalez, A.M., Korioth-Schmitz, B., Gelman, R.S., Montefiori, D.C., Haynes, B.F., Schmitz, J.E., 2015. Inhibitory effect of individual or combinations of broadly neutralizing antibodies and antiviral reagents against cell-free and cell-to-cell HIV-1 transmission. J. Virol. 89, 7813–7828.

Graphpad Prism, 2015. Equation: Sigmoidal dose-response (variable slope). http://www.graphpad.com/guides/prism/6/curve-fitting/index.htm?reg_classic_dr_variable.htm.

Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7, e1002158.

Gupta, P., Balachandran, R., Ho, M., Enrico, A., Rinaldo, C., 1989. Cell-to-cell transmission of human immunodeficiency virus type 1 in the presence of azidothymidine and neutralizing antibody. J. Virol. 63, 2361–2365.
Halper-Stromberg, A., Lu, C.L., Klein, F., Horwitz, J.A., Bournazos, S., Nogueira, L., Eisenreich, T.R., Liu, C., Gazumyan, A., Schaefer, U., Furze, R.C., Seaman, M.S., Prinjha, R., Tarakhovsky, A., Ravetch, J.V., Nussenzweig, M.C., 2014. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. Cell 158, 989–999.
Haynes, B.F., McElrath, M.J., 2013. Progress in HIV-1 vaccine development. Curr. Opin. HIV AIDS 8, 326–332.
Hessell, A.J., Rakasz, E.G., Poignard, P., Hangartner, L., Landucci, G., Forthal, D.N., Koff, W.C., Watkins, D.I., Burton, D.R., 2009. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection. PLoS Pathog. 5, e1000433.
Hessell, A.J., Rakasz, E.G., Tehrani, D.M., Huber, M., Weisgrau, K.L., Landucci, G., Forthal, D.N., Koff, W.C., Poignard, P., Watkins, D.I., Burton, D.R., 2010. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. J. Virol. 84, 1302–1313.
Hubner, W., McNerney, G.P., Chen, P., Dale, B.M., Gordon, R.E., Chuang, F.Y., Li, X.D., Asmuth, D.M., Huser, T., Chen, B.K., 2009. Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. Science 323, 1743–1747.
Ioannidis, J.P., Havlir, D.V., Tebas, P., Hirsch, M.S., Collier, A.C., Richman, D.D., 2000. Dynamics of HIV-1 viral load rebound among patients with previous suppression of viral replication. AIDS (London, England) 14, 1481–1488.
Janaka, S.K., Gregory, D.A., Johnson, M.C., 2013. Retrovirus glycoprotein functionality requires proper alignment of the ectodomain and the membrane-proximal cytoplasmic tail. J. Virol. 87, 12805–12813.
Jolly, C., Kashefi, K., Hollinshead, M., Sattentau, Q.J., 2004. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. J. Exp. Med. 199, 283–293.
Jolly, C., Mitar, I., Sattentau, Q.J., 2007. Adhesion molecule interactions facilitate human immunodeficiency virus type 1-induced virological synapse formation between T cells. J. Virol. 81, 13916–13921.
Julien, J.P., Sok, D., Khayat, R., Lee, J.H., Doores, K.J., Walker, L.M., Ramos, A., Diwanji, D.C., Pejchal, R., Cupo, A., Katpally, U., Depetris, R.S., Stanfield, R.L., McBride, R., Marozsan, A.J., Paulson, J.C., Sanders, R.W., Moore, J.P., Burton, D.R., Poignard, P., Ward, A.B., Wilson, I.A., 2013. Broadly neutralizing antibody PGT121 allosterically modulates CD4 binding via recognition of the HIV-1 gp120 V3 base and multiple surrounding glycans. PLoS Pathog. 9, e1003342.
Klein, F., Halper-Stromberg, A., Horwitz, J.A., Gruell, H., Scheid, J.F., Bournazos, S., Mouquet, H., Spatz, L.A., Diskin, R., Abadir, A., Zang, T., Dorner, M., Billerbeck, E., Labitt, R.N., Gaebler, C., Marcovecchio, P.M., Incesu, R.B., Eisenreich, T.R., Bieniasz, P.D., Seaman, M.S., Bjorkman, P.J., Ravetch, J.V., Ploss, A., Nussenzweig, M.C., 2012. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. Nature, 1–7.
Klein, F., Nogueira, L., Nishimura, Y., Phad, G., West, A.P., Halper-Stromberg, A., Horwitz Jr, J.A., Gazumyan, A., Liu, C., Eisenreich, T.R., Lehmann, C., Fätkenheuer, G., Williams, C., Shingai, M., Martin, M.A., Bjorkman, P.J., Seaman, M.S., Zolla-Pazner, S., Karlsson Hedestam, G.B., Nussenzweig, M.C., 2014. Enhanced HIV-1 immunotherapy by commonly arising antibodies that target virus escape variants. J. Exp. Med. 211, 2361–2372.
Ko, S.Y., Pegu, A., Rudicell, R.S., Yang, Z.Y., Joyce, M.G., Chen, X., Wang, K., Bao, S., Kraemer, T.D., Rath, T., Zeng, M., Schmidt, S.D., Todd, J.P., Penzak, S.R., Saunders, K.O., Nason, M.C., Haase, A.T., Rao, S.S., Blumberg, R.S., Mascola, J.R., Nabel, G.J., 2014. Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. Nature 514, 642–645.
Krakovska, O., Wahl, L.M., 2007. Costs versus benefits: best possible and best practical treatment regimens for HIV. J. Math. Biol. 54, 385–406.
Kwong, P.D., Mascola, J.R., Nabel, G.J., 2011. Rational design of vaccines to elicit broadly neutralizing antibodies to HIV-1. Cold Spring Harb. Perspect. Med. 1, a007278.
Lehmann, M.J., Sherer, N.M., Marks, C.B., Pypaert, M., Mothes, W., 2005. Actin- and myosin-driven movement of viruses along filopodia precedes their entry into cells. J. Cell Biol. 170, 317–325.
Little, S.J., Mclean, A.R., Spina, C.A., Richman, D.D., Havlir, D.V., 1999. Viral dynamics of acute HIV-1 infection. J. Exp. Med. 190, 841–850.
Malbec, M., Porrot, F., Rua, R., Horwitz, J., Klein, F., Halper-Stromberg, A., Scheid, J.F., Eden, C., Mouquet, H., Nussenzweig, M.C., Schwartz, O., 2013. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. J. Exp. Med. 210, 2813–2821.
Martin, N., Welsch, S., Jolly, C., Briggs, J.A., Vaux, D., Sattentau, Q.J., 2010. Virological synapse-mediated spread of human immunodeficiency virus type 1 between T cells is sensitive to entry inhibition. J. Virol. 84, 3516–3527.
Mascola, J.R., Haynes, B.F., 2013. HIV-1 neutralizing antibodies: understanding nature's pathways. Immunol. Rev. 254, 225–244.
Mascola, J.R., Stiegler, G., VanCott, T.C., Katinger, H., Carpenter, C.B., Hanson, C.E., Beary, H., Hayes, D., Frankel, S.S., Birx, D.L., Lewis, M.G., 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat. Med. 6, 207–210.
Massanella, M., Puigdomenech, I., Cabrera, C., Fernandez-Figueras, M.T., Aucher, A., Gaibelet, G., Hudrisier, D., Garcia, E., Bofill, M., Clotet, B., Blanco, J., 2009. Anti-gp41 antibodies fail to block early events of virological synapses but inhibit HIV spread between T cells. AIDS 23, 183–188.
Mazurov, D., Ilinskaya, A., Heidecker, G., Lloyd, P., Derse, D., 2010. Quantitative comparison of HTLV-1 and HIV-1 cell-to-cell infection with new replication dependent vectors. PLoS Pathog. 6, e1000788.
McCoy, L.E., Groppelli, E., Blanchetot, C., de Haard, H., Verrips, T., Rutten, L., Weiss, R.A., Jolly, C., 2014. Neutralisation of HIV-1 cell–cell spread by human and llama antibodies. Retrovirology 11, 83.
McDonald, D., Wu, L., Bohks, S.M., KewalRamani, V.N., Unutmaz, D., Hope, T.J., 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science 300, 1295–1297.
Moldt, B., Rakasz, E.G., Schultz, N., Chan-Hui, P.Y., Swiderek, K., Weisgrau, K.L., Piaskowski, S.M., Bergman, Z., Watkins, D.I., Poignard, P., Burton, D.R., 2012. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc. Natl. Acad. Sci. U. S. A. 109, 18921–18925.
Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Rüker, F., Katinger, H., 1993. A conserved neutralizing epitope on gp41 of Human-immunodeficiency-virus type-1. J. Virol. 67, 6642–6647.
Overbaugh, J., Morris, L., 2012. The antibody response against HIV-1. Cold Spring Harb. Perspect. Med. 2, a007039.
Parren, P.W., Marx, P.A., Hessell, A.J., Luckay, A., Harouse, J., Cheng-Mayer, C., Moore, J.P., Burton, D.R., 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J. Virol. 75, 8340–8347.
Pejchal, R., Doores, K.J., Walker, L.M., Khayat, R., Huang, P.S., Wang, S.K., Stanfield, R.L., Julien, J.P., Ramos, A., Crispin, M., Depetris, R., Katpally, U., Marozsan, A., Cupo, A., Maloveste, S., Liu, Y., McBride, R., Ito, Y., Sanders, R.W., Ogohara, C., Paulson, J.C., Feizi, T., Scanlan, C.N., Wong, C.H., Moore, J.P., Olson, W.C., Ward, A.B., Poignard, P., Schief, W.R., Burton, D.R., Wilson, I.A., 2011. A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. Science 334, 1097–1103.
Permanyer, M., Ballana, E., Badia, R., Pauls, E., Clotet, B., Este, J.A., 2012a. Trans-infection but not infection from within endosomal compartments after cell-to-cell HIV-1 transfer to CD4+ T cells. J. Biol. Chem. 287, 32017–32026.
Permanyer, M., Ballana, E., Ruiz, A., Badia, R., Riveira-Munoz, E., Gonzalo, E., Clotet, B., Este, J.A., 2012b. Antiretroviral agents effectively block HIV replication after cell-to-cell transfer. J. Virol. 86, 8773–8780.
Piguet, V., Steinman, R.M., 2007. The interaction of HIV with dendritic cells: outcomes and pathways. Trends Immunol. 28, 503–510.
Poignard, P., Sabbe, R., Picchio, G.R., Wang, M., Gulizia, R.J., Katinger, H., Parren, P.W., Mosier, D.E., Burton, D.R., 1999. Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo. Immunity 10, 431–438.
Putter, H., Heisterkamp, S.H., Lange, J.M.A., de Wolf, F., 2002. A Bayesian approach to parameter estimation in HIV dynamical models. Stat. Med. 21, 2199–2214.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Reh, L., Magnus, C., Schanz, M., Weber, J., Uhr, T., Rusert, P., Trkola, A., 2015. Capacity of broadly neutralizing antibodies to inhibit HIV-1 cell–cell transmission is strain- and epitope-dependent. PLoS Pathog., 11.
Ribeiro, R.M., Qin, L., Chavez, L.L., Li, D., Self, S.G., Perelson, A.S., 2010. Estimation of the initial viral growth rate and basic reproductive number during acute HIV-1 infection. J. Virol. 84, 6096–6102.
Rosenbloom, D.I.S., Hill, A.L., Rabi, S.A., Siliciano, R.F., Nowak, M.A., 2012. Antiretroviral dynamics determines HIV evolution and predicts therapy outcome. Nat. Med. 18, 1378–1385.
Rudnicka, D., Feldmann, J., Porrot, F., Wietgrefe, S., Guadagnini, S., Prevost, M.C., Estaquier, J., Haase, A.T., Sol-Foulon, N., Schwartz, O., 2009. Simultaneous cell-to-cell transmission of human immunodeficiency virus to multiple targets through polysynapses. J. Virol. 83, 6234–6246.
Rusert, P., Krarup, A., Magnus, C., Brandenberg, O.F., Weber, J., Ehlert, A.K., Regoes, R.R., Guenthard, H.F., Trkola, A., 2011. Interaction of the gp120 V1V2 loop with a neighboring gp120 unit shields the HIV envelope trimer against cross-neutralizing antibodies. J. Exp. Med. 208, 1419–1433.
Rusert, P., Mann, A., Huber, M., von Wyl, V., Günthard, H.F., Trkola, A., 2009. Divergent effects of cell environment on HIV entry inhibitor activity. AIDS 23, 1319–1327.
Russell, R.A., Martin, N., Mitar, I., Jones, E., Sattentau, Q.J., 2013. Multiple proviral integration events after virological synapse-mediated HIV-1 spread. Virology 443, 143–149.
Sagar, M., Akiyama, H., Etemad, B., Ramirez, N., Freitas, I., Gummuluru, S., 2012. Transmembrane domain membrane proximal external region but not surface unit-directed broadly neutralizing HIV-1 antibodies can restrict dendritic cell-mediated HIV-1 trans-infection. J. Infect. Dis. 205, 1248–1257.
Sanders, R.W., Venturi, M., Schiffner, L., Kalyanaraman, R., Katinger, H., Lloyd, K.O., Kwong, P.D., Moore, J.P., 2002. The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120. J. Virol. 76, 7293–7305.
Sattentau, Q., 2008. Avoiding the void: cell-to-cell spread of human viruses. Nat. Rev. Microbiol. 6, 815–826.
Scanlan, C.N., Pantophlet, R., Wormald, M.R., Ollmann Saphire, E., Stanfield, R., Wilson, I.A., Katinger, H., Dwek, R.A., Rudd, P.M., Burton, D.R., 2002. The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of alpha1->2 mannose residues on the outer face of gp120. J. Virol. 76, 7306–7321.
Schiffner, T., Sattentau, Q.J., Dorrell, L., 2013. Development of prophylactic vaccines against HIV-1. Retrovirology 10, 72.
Sherer, N.M., Lehmann, M.J., Jimenez-Soto, L.F., Horensavitz, C., Pypaert, M., Mothes, W., 2007. Retroviruses can establish filopodial bridges for efficient cell-to-cell transmission. Nat. Cell Biol. 9, 310–315.

Sigal, A., Kim, J.T., Balazs, A.B., Dekel, E., Mayo, A., Milo, R., Baltimore, D., 2012. Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. Nature 477, 95–98.

Sok, D., Doores, K.J., Briney, B., Le, K.M., Saye-Francisco, K.L., Ramos, A., Kulp, D.W., Julien, J.P., Menis, S., Wickramasinghe, L., Seaman, M.S., Schief, W.R., Wilson, I.A., Poignard, P., Burton, D.R., 2014. Promiscuous glycan site recognition by antibodies to the high-mannose patch of gp120 broadens neutralization of HIV. Sci. Transl. Med. 6, 236ra63.

Sowinski, S., Jolly, C., Berninghausen, O., Purbhoo, M.A., Chauveau, A., Kohler, K., Oddos, S., Eissmann, P., Brodsky, F.M., Hopkins, C., Onfelt, B., Sattentau, Q., Davis, D.M., 2008. Membrane nanotubes physically connect T cells over long distances presenting a novel route for HIV-1 transmission. Nat. Cell Biol. 10, 211–219.

Titanji, B.K., Aasa-Chapman, M., Pillay, D., Jolly, C., 2013. Protease inhibitors effectively block cell-to-cell spread of HIV-1 between T cells. Retrovirology 10, 161.

Trkola, A., 2014. HIV: potency needs constancy. Nature 514, 442–443.

Trkola, A., Kuster, H., Rusert, P., Joos, B., Fischer, M., Leemann, C., Manrique, A., Huber, M., Rehr, M., Oxenius, A., Weber, R., Stiegler, G., Vcelar, B., Katinger, H., Aceto, L., Günthard, H.F., 2005. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat. Med. 11, 615–622.

Trkola, A., Kuster, H., Rusert, P., von Wyl, V., Leemann, C., Weber, R., Stiegler, G., Katinger, H., Joos, B., Gunthard, H.F., 2008. In vivo efficacy of human immunodeficiency virus neutralizing antibodies: estimates for protective titers. J. Virol. 82, 1591–1599.

Trkola, A., Purtscher, M., Muster, T., Ballaun, C., Buchacher, A., Sullivan, N., Srinivasan, K., Sodroski, J., Moore, J., Katinger, H., 1996. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. J. Virol. 70, 1100–1108.

Walker, L., Phogat, S., Chan-Hui, P., Wagner, D., Phung, P., Goss, J., Wrin, T., Simek, M., Fling, S., Mitcham, J., Lehrman, J., Priddy, F., Olsen, O., Frey, S., Hammond, P., Kaminsky, S., Zamb, T., Moyle, M., Koff, W., Poignard, P., Burton, D., 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326, 285–289.

Walker, L.M., Burton, D.R., 2010. Rational antibody-based HIV-1 vaccine design: current approaches and future directions. Curr. Opin. Immunol. 22, 358–366.

Walker, L.M., Huber, M., Doores, K.J., Falkowska, E., Pejchal, R., Julien, J.P., Wang, S.K., Ramos, A., Chan-Hui, P.Y., Moyle, M., Mitcham, J.L., Hammond, P.W., Olsen, O.A., Phung, P., Fling, S., Wong, C.H., Phogat, S., Wrin, T., Simek, M.D., Protocol G Principal Investigators, Koff, W.C., Wilson, I.A., Burton, D.R., Poignard, P., 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature 477, 466–470.

Wibmer, C.K., Bhiman, J.N., Gray, E.S., Tumba, N., Abdool Karim, S.S., Williamson, C., Morris, L., Moore, P.L., 2013. Viral escape from HIV-1 neutralizing antibodies drives increased plasma neutralization breadth through sequential recognition of multiple epitopes and immunotypes. PLoS Pathog. 9, e1003738.

Yen, P.J., Herschhorn, A., Haim, H., Salas, I., Gu, C., Sodroski, J., Gabuzda, D., 2014. Loss of a conserved N-linked glycosylation site in the Simian immunodeficiency virus envelope glycoprotein V2 region enhances macrophage tropism by increasing CD4-independent cell-to-cell transmission. J. Virol. 88, 5014–5028.

Zhong, P., Agosto, L.M., Ilinskaya, A., Dorjbal, B., Truong, R., Derse, D., Uchil, P.D., Heidecker, G., Mothes, W., 2013. Cell-to-cell transmission can overcome multiple donor and target cell barriers imposed on cell-free HIV. PLoS One 8, e53138.

Zhong, P., Agosto, L.M., Munro, J.B., Mothes, W., 2013b. Cell-to-cell transmission of viruses. Curr. Opin. Virol. 3, 44–50.